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VOLUME 5 NUMBER 3

NUTRITION REVIEWS

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NUTRITION REVIEWS

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THE NUTRITIONIST'S INTEREST IN SOILS AND AGRICULTURE

Graham Lusk's definition of nutrition as "The sum of the processes concerned in the growth, maintenance, and repair of the living body as a whole or of its constituent organs" ("The Science of Nutrition," *4th Ed., Saunders, Philadelphia, p. 75 (1928)*) has given way in more recent years to a broader one including the composition of the substances acted upon by the body (foodstuffs) and the science of the production of these foodstuffs. Sir Cedric Stanton Hicks (*New Zealand Med. J.* **45**, *190 (1946)*) has redefined nutrition as "A continuous flow of material from the soil and back to the soil." Numerous examples of this broadening viewpoint in nutrition come to mind. Perhaps none is more striking than the organization of the Food and Agriculture Organization of the United Nations (FAO) (*Nutrition Reviews* **4**, *8 (1946)*). The fundamental relationship of nutrition to agricultural and soil programs has been well epitomized by the United Nations Interim Commission on Food and Agriculture ("The Work of FAO," *Washington, D. C., p. 23 (1945)*) "Over those parts of the earth not covered by water lies a thin crust of soil, perhaps equal by comparison to the thickness of a sheet of tissue paper wrapped around a globe six feet through. Much of this soil is inaccessible for cultivation, or it is unusable for other reasons. From the rest, the world's growing population, now more than 2,000 million, must draw all their sustenance except what they get from the sea; and even the fishes, like all other living things, are fed in the final analysis out of the fertility of the land. Whether this thin layer of soil is to be a wasting asset or one maintained in perpetuity and made more fruitful for mankind will depend on how it is used and managed. Nothing more deeply concerns the well-being of men and nations. FAO is dedicated to furthering good use and good management, in all ways and by all peoples, of this most basic of man's resources."

Sir Cedric Hicks (*loc. cit.*) points out that since human nutrition may be considered as a flow of substance within a closed cycle, one may, therefore, determine how much human life may be supported by a given area of land. He further notes that this closed cycle is oftentimes broken because of modern man's development of towns and cities. Certain data, namely those of East, indicate that some 40 per cent of the global surface is available arable land and that these 13,000 million acres will ultimately have to be balanced against the concentration of human population which they will support. Quoting the same authority, Hicks indicates that to support an individual at the nutritional level of that of persons in the United States it is necessary to have some 3.5 acres per person per year in agriculture. Further, to support a man at the European peasant level, 2.5 acres per person per year are necessary. Assuming, therefore, an increase of 1 per cent per annum in population, he indicates that for the United States alone the maximum supportable figure for population is 160 million, which number would be attained between the years 1950 and 2000. In approximately that period of time the American population would presumably become self limited. It is further indicated that as the population of a country approaches the density of one person per 2.5 acres of arable land, the tendency is actually for a decline in total population. Therefore, if one divides the actual arable acres per person available in a given country by 2.5 acres, one may derive what may be termed a population saturation

coefficient. If this saturation coefficient is unity, there is balance between the soil and the population. If it is less than unity, it may be argued that the land cannot maintain the population pressure and food must be imported. Using this method of indicating a saturation coefficient, Hicks has tabulated the relative population densities of a number of countries. It is of interest that in a total of 27 countries only 6 countries have a population coefficient greater than 1.0. Of these, Canada, Argentina, and New Zealand have very large population coefficients and Russia, United States, and Australia have population coefficients above the level of 1.24. Such figures give us pause to reflect whether it might be possible for the total population of the world to attain a highly desirable nutritional level.

Hicks has further considered whether it might be possible to restore balance between the inhabitants and the productivity of the soil in those areas in which the population pressure is greater than one person per 2.5 acres. He suggests three possibilities for bringing about a more rapid and larger flow of nutrient material from the soil. These may be enumerated as: (1) Continuous cutting of the fodder crops to remove the young shoots of the plant which presumably are richest in nutrient material. (2) Use of water culture or hydroponics. (3) Introduction of new strains (agrotypes) with shorter crop cycles which improve the annual possible turnover per acre, and, furthermore, enable a given agrotype to be grown in latitudes where otherwise there had been insufficient solar energy to complete the crop cycle. In a much less concrete, and, hence, less satisfactory manner, Hicks (*loc. cit.*) discusses the role of natural fertilizers, humus, and compost in determining the quality of crops produced on the soil. Although these factors are undoubtedly of utmost importance in the soil, it is doubtful that they play the rather mystic role assigned to them by Hicks.

Another discussion of soil and man's food supply is that of F. A. Pearson and F. A. Harper ("The World's Hunger," *Cornell University Press, Ithaca (1945)*). These writers point out that there are seven factors which must exist in proper combination so that land may be arable. These factors are: a favorable topography, adequate sunlight and carbon dioxide, favorable temperature, reliable rainfall, fertile soil, and adequate rainfall. It is emphasized that although 43 per cent of the land surface of the earth has adequate rainfall for crop production, a majority of this land cannot be considered arable because the rain does not come at the right time of the year, it is not dependable, the temperature is not favorable, the soil is poor, or the soil does not possess the correct topography. Similarly, if one considers temperature alone, over four fifths of the earth's surface might be judged satisfactory for crop production. Some two thirds of this area is satisfactory from the standpoint of topography and almost one-half from the standpoint of soils. Finally, when a combination of all of these factors is considered it is apparent that only 7 per cent of the land surface of the earth is adaptable to agricultural production; that is, a total of 2580 million acres. After arriving at this figure these authors caution "Although unforeseen new technics of food production may be developed, those who would plan for the future of the world had best be conservative as to the prospects for expansion. In the main, expansion of the world's agriculture beyond the area now being used is a hazardous undertaking. The requirements of nature for food production are so rigid that thus far man has been able to use only a small proportion of the earth's land surface and has been defeated in most of his attempts to extend his boundaries." This caution is especially pertinent when there is such apparent diversity of ideas, as revealed in these two papers, as to the extent of the total arable land.

The Cornell workers next consider the question of increasing production on the present acreage. They first note that the world produces about half a ton of food per

acre. This production is remarkably constant whether one considers the high yielding corn areas with highly mechanized agriculture of North America or the high yielding rice crop with small farms and manual labor with little machinery of Asia. Although it is not possible to review all of their arguments, it is well to note certain points which are made. Thus, it is noted that additional use of animal manures is not likely to increase crop yields to any considerable extent because expansion of the present livestock production would of necessity increase the acreage of hay and pasture at the expense of grains, and this is unlikely to occur on any great scale throughout the world. Likewise, the use of green manure crops—which it is well established can increase the yields of a given acre—is restricted as a method of increasing total food supply because of the amount of human, animal, and mechanical power required to fit the land, plant the crop, and plow it under, and because of the difficulty of fitting such crops into rotation without destroying food production. These workers note that mechanization increases food supplies from a given area, largely due to the decrease in the amount of food fed to horses and other animals previously used for power. They conclude that mechanization will increase the world's food supply, but not nearly so much as many persons believe. In agreement with Hicks (*loc. cit.*), it is noted that new and improved varieties of crops will undoubtedly add considerably to man's food supply. However, in disagreement with Hicks, these writers feel that double-cropping will not materially increase food production, largely because few areas in the world have a sufficiently long growing season, or the abundance of rainfall, or water for irrigation necessary for the double-cropping procedures. In summary, Pearson and Harper conclude "Food production can be materially expanded on the present crop area by more complete conservation of animal manures, the expansion of commercial fertilizers, the use of more machinery, more efficient livestock, new and improved varieties of crops, control of animal and plant diseases, and more double-cropping. These methods seem to hold more promise for increasing the world's food supply than does an expansion of acreage. Changes, however, will be slow and gradual, and with little likelihood that they will contribute much during any one generation. In fact, the expansion will probably be less than the growth of the population. This forecast is based on our present knowledge. . ."

Although some of the data upon which the analyses by Hicks and by Pearson and Harper are based are at variance, both analyses lead to the same conclusion: So long as our food supply depends upon the type of agriculture in existence at this time, the number of people which the world's food supply will support will depend upon the standard of living of those people. Whether the science of nutrition can ultimately provide means of supporting an expanded world population at a very high nutritional plane through developments of new methods of food production, such as the production of food yeasts and of synthetic foodstuffs, remains for the future to decide. At any rate, the scientist interested in nutrition has a definite link with the agricultural scientist and the soil scientist. It is encouraging to see the obliteration of the boundaries between these fields in common efforts to provide the best possible nutritional level for the greatest number of persons. It is imperative, however, for those in each area of this broad field to have available and understand reliable facts from the other areas. For example, at least one of the estimates of arable land quoted above must be wrong. Nutritionists would be interested in learning a reliable estimate of maximum arable land.

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PRESENT KNOWLEDGE OF THE RELATION OF NUTRITION TO DENTAL CARIES

Dental caries has plagued the human race for centuries but is still one of the least understood diseases, largely because dental teaching and research have concentrated on the repair of tooth decay rather than on its prevention. The concept that a relationship exists between diet and tooth decay has been widely accepted, but the more intimate details of this relation are not known. A large amount of opinion supports the concept that local conditions in the oral cavity constitute the controlling influence, but this simple concept neglects much data on the effect of variations in internal metabolism on the incidence and progress of carious lesions. In investigations on the effects of diet on tooth decay, the oral and systemic environments of the teeth should be considered. After eruption the oral environment of the teeth is a result of the salivary secretions, the oral flora, and the diet, while the digestive and assimilated portion of the diet controls the systemic environment of the teeth, and in addition may modify the type and quantity of the salivary secretions.

Numerous surveys of primitive races have shown that a change of diet so as to include foods of civilization results in an increase of caries. It has been assumed that an inadequate diet results from this change. Natural differences in native diets may also result in striking differences in caries incidence. For example, J. B. Orr and J. L. Gilks (*Med. Res. Council (Great Britain), Special Report Ser. no.* **155** (*1931*)) made a survey of the health of two African tribes, the Masai and the Kikuyu. The diet of the Masai consisted largely of milk, meat, and raw blood, while that of the Kikuyu was composed mainly of cereal with some roots and fruits. Of 254 Masai, 1.6 per cent of the boys and 3.6 per cent of the girls had carious lesions. Among the 2500 Kikuyus the incidence of carious lesions was 13.7 per cent for the boys and 13.1 per cent for the girls. Rickets was observed in 63 per cent of the Kikuyu children but was said to be rare among the Masai.

The effect of adequate diets upon the dental caries incidence in growing children has been investigated by J. D. Boyd and C. L. Drain (*Nutrition Reviews* **2**, *35* (*1944*)) who observed a very low incidence of new carious lesions and an arrest of old lesions in diabetic children who had been maintained on excellent diets under strict supervision. The main constituents of the diet provided were: milk, cream, butter, eggs, meats, vegetables, and fruits, supplemented with adequate amounts of cod liver oil. The investigators postulated that the arrest of tooth decay was promoted by the correction of nutritional deficiencies. When an orphanage diet was supplemented with milk, butter, and eggs in sufficient amounts to make the diet adequate, an arrested or stationary dental caries activity was observed in 42 of the 53 children during the eight month observation period (C. L. Drain and J. D. Boyd, *J. Am. Dent. Assn.* **22**, *155* (*1935*)). In experiments to determine if recommendations to the mothers of outpatient children for the improvement of the children's diet would have any effect upon the dental caries incidence, P. R. Howe, R. L. White, and M. D. Elliott (*Ibid.* **29**, *38* (*1942*)) and P. R. Howe, R. L. White, and M. Rabine (*Am. J. Dis. Child.* **46**, *1045* (*1933*)) observed that there was a much lower incidence of new carious lesions in the children who were given dietary instructions than in those who were given no advice.

Of the various nutrients, vitamin D has been most thoroughly investigated with regard to dental lesions. Lady M. Mellanby was one of the first to postulate a beneficial effect upon the addition of vitamin D to children's diets. Several of her early experiments were performed in the early 1920's when nutritional knowledge was very incomplete; since her early experimental regimens included variable factors in addi-

tion to vitamin D, interpretation of the results are difficult. In the latest and best controlled of Mellanby's studies, the effect of an olive oil supplement to the orphanage diet was compared with a supplement of olive oil which contained about 9000 units of irradiated ergosterol per day (*Med. Res. Council (Great Britain), Special Report Ser. no.* **211** *(1936)*). The children in both groups were housed in the same institution and had the same environment. During the observation period of eighteen months, the group of children which received vitamin D had a significantly lower incidence of new carious lesions than the control group. Numerous other groups of investigators have tested the effect of vitamin D since the publication of Mellanby's original reports. The majority of these studies have confirmed the observations just mentioned; the most notable exception being the study of C. D. M. Day and H. J. Sedwick (*Dental Cosmos* **77,** *442 (1935)*) in which 40,000 units of vitamin D per day did not result in a decrease in the dental caries incidence below that in the control group. Such a massive dose actually may have been sufficiently toxic to disturb the calcium and phosphorus metabolism of the experimental group instead of improving it.

The experimental data on the relation of vitamin D to tooth decay are strengthened again by survey data obtained in the United States on the relation of latitude and of annual hours of sunshine to the dental caries incidence. C. A. Mills (*J. Dent. Research* **16,** *417 (1937)*) found that there was an increase in caries experience with an increase in latitude of roughly fifteen more decayed, missing, or filled teeth per 100 children for each added degree of latitude, which amounted to well over a 200 per cent increase from the Gulf of Mexico to the Canadian border. B. R. East (*Am. J. Pub. Health* **29,** *777 (1939)*) has analyzed the relation between the annual hours of sunshine and the dental caries experience and observed a definite decrease in the caries experience with an increase in the amount of sunshine, with roughly a 4 to 5 per cent decrease in the dental caries rate with each additional 100 hours of sunshine per year.

Vitamin D would be expected to be beneficial through the maintenance of normal calcium and phosphorus metabolism. J. D. Boyd, C. L. Drain, and G. Stearns (*J. Biol. Chem.* **103,** *327 (1933)*) observed a statistically significant relationship between daily calcium and phosphorus retentions and dental caries activity with a lower retention of both in children with active dental caries. When the retention of calcium and phosphorus was improved by dietary means, there was a concurrent reduction in the dental caries activity (C. L. Drain and J. D. Boyd, *J. Am. Dent. Assn.* **22,** *155 (1935)*). In contrast, E. H. Siegel, L. M. Waugh, and M. Karshan (*Am. J. Dis. Child.* **59,** *19 (1940)*) concluded from two day calcium and phosphorus balance trials made in three Eskimo communities that there was no relation between calcium and phosphorus retentions and dental caries activity. The large positive and negative retention values reported may be true observations for the two day balance periods, but they could not exist for long periods without serious consequences.

No conclusive evidence has been presented that other vitamins bear any special relation to caries incidence. Although vitamin A deficiency has a deleterious effect on the histodifferentiation of the odontogenic epithelium in the incisor of rodents, and ascorbic acid is essential for normal periodontal structures and calcification of dentin in the guinea pig (*Nutrition Reviews* **4,** *41 (1946)*), the deficiency of these vitamins has not been demonstrated to have an effect on human dental caries. Caries incidence seems not to be elevated in deficiencies of the vitamin B-complex (A. H. Kniesner, A. W. Mann, and T. D. Spies (*J. Dent. Research* **21,** *259 (1942)*), although the importance of the vitamin B-complex in maintenance

of the soft oral structures is not to be overlooked (*Nutrition Reviews* **4**, *41* (*1946*)).

Two reports on the effect of synthetic vitamin K in chewing gum on the incidence of tooth decay have been reported (*Nutrition Reviews* **4**, *213* (*1946*)). The conclusion of the one report indicated a positive relationship but the published data did not give strong support to the conclusion. The other investigation reported no relationship. Large supplements of synthetic vitamin K were found to be ineffective in reducing the incidence of carious lesions in the white rat.

Two interesting relationships have been observed between the public water supplies of various communities and the dental caries experience of the citizens. In regions where the drinking water is hard, there is a lower incidence of carious lesions than where the water is soft (C. A. Mills, *J. Dent. Research* **16**, *417* (*1937*)). Likewise where the drinking water contains 1 part per million of fluorine or above, the children consuming this water during the period of tooth development have a significantly lower incidence of carious lesions than those children who grew up in regions where the fluorine content of the drinking water was less than 1 p.p.m. (*Nutrition Reviews* **1**, *182* (*1943*)). The effect of the addition of enough fluorine to a public water supply to make a total of 1 p.p.m. fluorine is being studied in Newburgh, N. Y. and in other cities to determine if this is a possible method for a partial mass control of dental caries (*Ibid.* **3**, *115* (*1945*)). Most studies of the effect of either the hardness of the drinking water supply or its fluorine content have been carried on independently of each other. One extensive survey in which the two factors were considered simultaneously has been described by T. Ockerse (*South African Med. J.* **18**, *255* (*1944*)). His data indicated that there is an inverse relationship between dental caries incidence and the hardness of the drinking water, independent of the fluorine content and likewise an inverse relationship between the dental caries incidence and the fluorine content of the water, independent of the hardness of the water. The effect of fluorine-containing waters has been found to be greatest during the period of tooth development before eruption and in the maturation process shortly after eruption. This would indicate that the effect of the fluorine-containing waters was at least partially nutritional and could not be explained solely on the basis of the oral environmental contact with the teeth. The final test of the value of fluorine in reducing the incidence of tooth decay must await the results of those investigations where fluorine compounds are being added to different water supplies. The answer to the question of the possible toxic effects of the addition of 1 p.p.m. of fluorine to any public water supply will be obtained simultaneously.

There has been much discussion of the possible role of sugar and other refined carbohydrate foods in the production of dental caries. In the few recorded observations in which large quantities of sugar were incorporated into a fully adequate diet, J. D. Boyd, C. L. Drain, and M. V. Nelson (*Am. J. Dis. Child.* **38**, *721* (*1929*)) did not observe any new carious lesions and those active at the beginning were arrested. It is noteworthy that these children received as high as 60 per cent of their calories in the form of dextrose for a period of months. However, this diet contained no starch or cereals to hinder clearance of the soluble dextrose from the oral cavity. The experiment of M. Koehne, R. W. Bunting, and E. Morrell (*Ibid.* **48**, *6* (*1934*)) is frequently discussed as an example of the addition of sugar to an adequate diet. The authors reiterated the statement that the nutritional adequacy of the diet had been carefully maintained; however, it was stated that no vitamin D supplement was given during the eighteen month period of hospitalization. Evidence that the calcium and phosphorus metabolism of these children was not entirely normal was provided in the low calcium and

phosphorus retentions recorded. Therefore the conclusion of Koehne and co-workers that the addition of sugar to an adequate diet had increased the susceptibility to dental caries cannot be justified in view of these data.

The studies of Boyd and Drain which concerned the relation of an adequate diet to dental caries (*loc. cit.*) have been interpreted in terms of oral environmental effects by other investigators. The diet of Boyd and Drain was one which contained a low amount of carbohydrate since it had been designed primarily for the control of diabetes. Therefore oral environmentalists felt that the data obtained were manifestations of the low carbohydrate content of the diet and not of the adequacy of the diet. To test this hypothesis, J. D. Boyd (*Nutrition Reviews* **3,** *160* (*1945*)) compared their original diet with one which offered only half as much fat and twice the amount of carbohydrate, without reducing the adequacy of the diet in other nutritional essentials. This study showed no constant or significant difference in the average rate of progression of carious lesions nor in the appearance of new lesions in the two groups of children maintained on the two diets for long periods. Therefore Boyd's conclusion was that the protection from acute carious lesions depended on the nutritive value of the diet rather than its carbohydrate-fat ratio. The relative effects of oral environment and of over-all nutritive value of a diet upon the dental caries incidence need much more thorough investigation, especially in so far as the sugar content of adequate diets is concerned.

From a strictly nutritional standpoint, the value of adequate vitamin D and the maintenance of an adequate retention of calcium and phosphorus have been demonstrated to aid in the maintenance of a low incidence of tooth decay, even during periods of most rapid growth when metabolic demands are at their greatest levels. The fluorine content of drinking water may be another factor which influences dental caries incidence. Every effort should be made to apply these facts to nutritional programs with the knowledge that definite beneficial results on the dental caries incidence can be expected.

MICROCHEMICAL ANALYSIS

Preliminary accounts of the methods and apparatus of microchemical analysis which can be carried out on fractions of a milliliter of material have been reported (*Nutrition Reviews* **3,** *336* (*1945*); **4,** *141* (*1946*)).

O. H. Lowry and T. H. Hunter (*J. Biol. Chem.* **159,** *465* (*1945*)) described in detail a method for the determination of plasma proteins, using a density gradient tube with plasma samples of 2 to 3 cu.mm. volume. The method of collecting the blood in melting-point capillaries, and centrifuging the tubes for separation of the plasma from the cells, was described. Blood could be obtained from the finger or ear, and similar results were obtained in macro or micro samples of venous blood, or with blood from ear or finger puncture. The results were repeated easily with considerable precision. Comparison of the results obtained by this method with those obtained on the same blood samples by the macro Kjeldahl digestion procedure showed that only 5 per cent of results differed by more than ± 0.4 g. per cent from the Kjeldahl method results. The standard deviation of the difference from the Kjeldahl method results was ± 0.24 g. per cent.

The effect of storage on the blood samples was studied, and at 30°C. the apparent protein content of the plasma increased by about 1 g. per cent after twenty-five hours, but at 4°C. there was no significant increase in forty-eight hours. This observation shows

that for this estimation it is permissible to collect samples in the field and to mail or dispatch them to the laboratory, provided they are refrigerated.

O. H. Lowry, J. A. Lopez, and O. A. Bessey (*J. Biol. Chem.* **160,** *609 (1945)*) described the determination of ascorbic acid in 0.01 ml. of blood serum using the method of J. H. Roe and C. A. Kuether (*Ibid.* **147,** *399 (1943)*) with dinitrophenylhydrazine. This method agreed precisely with results obtained by the macromethod of R. L. Mindlin and A. M. Butler (*Ibid.* **122,** *673 (1938)*) who titrated the plasma against a solution of 2,6-dichlorophenolindophenol.

Of general significance to the field of microchemical analysis is the adaptation by O. H. Lowry and O. A. Bessey (*J. Biol. Chem.* **163,** *633 (1946)*) of the Beckman spectrophotometer for very small amounts of liquids. This promises to make possible the use of a variety of colorimetric methods now available on the macro scale in a micro form. The basic feature in converting such methods to a micro scale is to maintain the approximate concentrations of reactants employed in the macro methods, but to work with greatly reduced volumes. The resulting small volumes must be accommodated in microcuvettes but with the usual length of light path in order not to lose the advantage of the smaller volumes employed. The cuvette has a width of only 2 mm., so that with the standard length of 10 mm. it can be filled to a height of about 2.5 mm. with 50 cu.mm. of solution. By placing a diaphragm between the light exit slit of the photometer and the cuvette, it is possible to direct a light path only 2 × 2 mm. in cross section through the cuvette without the light impinging on the cuvette walls of the meniscus of the solution.

Such adaptation of the Beckman instrument calls for an accurately made diaphragm and special care in the maintenance of the precise position of both cuvette and diaphragm in the spectrophotometer. Directions for the use of the cuvettes and diaphragms are given by the authors. Since the area of the light beam is so much reduced by the diaphragm, it is sometimes necessary to use the Beckman spectrophotometer with its maximum electrical sensitivity.

Figures and curves are given to demonstrate the precision of the micro measurements. They were obtained with solutions of inorganic phosphate treated with a modified Fiske and Subbarow reagent to give a blue color. Readings were made at 870 millimicrons and the optical density was shown to vary with considerable precision in direct proportion to the concentration of the phosphate. The readings were reproducible with precision, and were closely similar to those obtained with larger volumes of solution in a macrocuvette.

The absorption curve of the solution was taken using both the adapted cuvettes of 1 mm. width, holding 0.025 cu.mm. solution and the ordinary cuvettes containing 3 ml. of solution. Although the macrocuvettes were used with a spectral band width of 0.6 to 1.2 millimicrons, while the adapted cuvettes required the use of a band width of 5 to 10 millimicrons on account of the smaller cross section of the light path, the two curves obtained were identical within very small limits of deviation. Illustrative data were also presented of the absorption spectrum of phenylalanine, which has a number of narrow bands between 245 and 270 millimicrons. These bands were suppressed when using a cuvette 1 mm. wide, on account of the increased slit width required for sufficient light with resulting decrease in the purity of the monochromatic light. Thus the fine structure of bands cannot at present be determined on the micro scale, but no impairment of the accuracy of quantitative comparisons at any given slit setting occurs.

A method for the rapid determination of serum alkaline phosphatase has also been described by O. A. Bessey, O. H. Lowry, and M. J. Brock (*J. Biol. Chem.* **164,** *321 (1946)*). This does not use such small volumes of solution for spectrophotometry and can be

carried out with any instrument capable of adaptation to 0.5 ml. volumes. The substrate chosen for measuring the amount of the enzyme was p-nitrophenylphosphate, which has been used in the past by a few workers. This substance is colorless but has a maximum absorption at 310 millimicrons whereas the p-nitrophenolate set free by the phosphatase is yellow with a maximum absorption at 400 millimicrons. Acidification of the solution after the phosphatase action converts the p-nitrophenolate produced to free p-nitrophenol, thereby abolishing the absorption at 400 millimicrons and providing a blank for determining the absorption at 400 millimicrons of the serum itself. The proposed method of estimation, employing 5 to 20 cu.mm. of serum, involves incubation of the serum for thirty minutes with a measured volume of a freshly prepared p-nitrophosphate reagent buffered at pH 10.3 to 10.4, and subsequent measurement of the light extinction at 400 millimicrons.

The authors were able to analyze 50 to 100 serums in two hours, showing the rapidity of assay. The results were closely proportional to those obtained on the same serums by the method of A. Bodansky (*J. Biol. Chem.* **101,** *93 (1933)*). Results were expressed in p-nitrophenyl phosphate units, which had to be multiplied by the factor 1.79 to give the Bodansky units. The slight divergence of results by the two methods is illustrated by the fact that the standard deviation of single determinations of this factor was only ± 0.10.

Evidence was also presented to show that for human phosphatase studies it is unnecessary to consider the immediate dietary history, since the phosphatase level was found to be unaffected by various dietary conditions.

DIETARY TREATMENT OF INFECTIOUS HEPATITIS

The beneficial effect of a high protein diet in the treatment of infectious hepatitis has been recognized (*Nutrition Reviews* **4,** *168 (1946)*) but it has been the general practice to exclude as much fat as possible from these diets. The belief that fat is valueless and even injurious in patients with acute inflammatory liver disease developed from the earlier teaching that an impairment of hepatic biliary function occurred and interfered with the digestion and absorption of fat. More recent experimental studies on the relation of dietary fat to the liver pathology produced on diets low in protein and deficient in lipotropic factors have been interpreted as supporting the idea that tolerance for fat must necessarily be low in liver disease, such as infectious hepatitis. Evidence to the contrary has been reported by C. L. Hoagland, D. H. Labby, H. G. Kunkel, and R. E. Shank (*Am. J. Pub. Health* **36,** *1287 (1946)*) who have compared the effects of high and low fat, high protein diets on the recovery rate of patients with infectious hepatitis. Fat appeared to be well utilized in the subjects on the high fat, high protein diet and the resulting high caloric intake was associated with a more rapid restoration of normal hepatic activity. These observations are in agreement with the present belief that the liver parenchyma and not the major bile passages are involved in the disease (B. Lucké, *Am. J. Path.* **20,** *471 (1944)*). Furthermore, it is well established that a high protein intake provides an extra source of the lipotropic amino acid, methionine.

Seventy patients were studied; 37 received a diet containing 150 g. of fat, and 30 received a diet containing 50 g. The protein intake was 150 g. in both groups. Carbohydrates were unrestricted and each patient was encouraged to eat the maximum amount of food compatible with comfort and appetite. The extra fat was derived largely from milk, cream, butter, and eggs.

The high fat diet resulted in a much greater caloric intake because of its improved palatability and smaller bulk. The food consumption in the high fat group ranged from 2400 to 3500 calories whereas that in the low fat group varied from 2100 to 2700 calories. Anorexia during the first few days of hospitalization was more effectively overcome by use of the high fat food mixture. This result was believed to be of considerable importance because the caloric intake after onset of the disease falls far below the maintenance requirement unless special measures are used to increase the consumption of food. The observations of S. Goldschmidt, H. M. Vars, and I. S. Ravdin (*J. Clin. Invest.* **18,** *277 (1939)*) on chloroform poisoning and of A. J. Patek (*Bull. N. Y. Acad. Med.* **19,** *498 (1943)*) on chronic liver insufficiency support the conclusion that extra protein, supplied before and during the stage of greatest liver damage, significantly protects this organ against more severe injury. Patients in the high fat group gained in weight as a result of the voluntary consumption of additional food. Similar gains did not occur in the low fat group and there was no increase at all for two weeks after the institution of the diet.

Improved appetite and the resulting increased caloric and protein intake were associated with clinical recovery, as measured by the bromsulfalein liver function test. Average results obtained on 30 patients (15 in each group) in whom dietary control was begun within the first week of the disease, showed that normal bromsulfalein values were obtained on the twenty-fifth and thirty-third days of convalescence on the high and low fat diets, respectively. These intervals were appreciably less than in other patients in whom dietary treatment was not started until later in the course of the disease. The data emphasized that the value of the high protein regimen is noticeably greater if it is instituted as soon as possible after the appearance of the infection.

The study of Hoagland *et al.* offers no support for direct, favorable influence of fat on the restoration of normal liver function in infectious hepatitis. Its beneficial effect was indirect and was attributed to the patient finding it easier to eat more of the high fat than of the low fat diet. It is possible that recovery would be accelerated even more if it were possible to attain the desired intake of protein and of calories by supplying a food mixture moderately low in fat. This would require the preparation of particularly acceptable meals and the skillful persuasion of the patients to eat more food.

Additional information must be acquired before it is possible to describe accurately the most effective diet in liver disease. It would be highly advantageous to replace the terms, "high protein" or "high fat," with specific references to protein on the basis of its content of essential amino acids, to fat on the basis of molecular weight and degree of saturation, and to lipotropic and antilipotropic factors on the basis of dietary sources of choline, inositol, cholesterol, and other relevant compounds.

MATERNAL DIET AND HYDROCEPHALUS IN RATS

In recent years several relations between the diet of the mother and abnormal fetal development have been recognized and described. J. Warkany and co-workers observed marked congenital deformities characterized by intrauterine resorption and skeletal malformities in the young rat fetus which were related to the riboflavin and vitamin D content of the maternal diet (*Nutrition Reviews* **1,** *49 (1942)*; **1,** *207 (1943)*; **2,** *157, 297 (1944)*). O. B. Ross *et al.* (*Ibid.* **1,** *49 (1942)*) produced serious

reproductive failures in swine and rats when a practical ration of yellow corn, soybean oil meal, ground alfalfa hay, iodized salt, and calcium carbonate was fed but when the alfalfa content of the diet was increased from 5 to 15 per cent, there was a reduction in the reproductive difficulties. In later studies, O. B. Ross, P. H. Phillips, G. Bohstedt, and T. J. Cunha (*J. Animal Sci.* **3,** *406 (1944)*) described various deformities which were observed in the offspring of sows fed this basal ration. These deformities were characterized by a growing together of the toes, clubfeet, and shaking palsy.

A further example of the effect of the maternal diet upon fetal development has been presented by L. R. Richardson and A. G. Hogan (*J. Nutrition* **32,** *459 (1946)*). One group of female albino rats was fed a purified ration composed of: acid-washed and alcohol-extracted casein 30 g., cerelose 52 g., wood pulp 3 g., salt mixture 5 g., and lard 10 g., adequately supplemented with choline chloride, inositol, para-aminobenzoic acid, thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, calcium pantothenate, niacin, biotin, vitamin A, vitamin D, alpha-tocopherol, and 2-methyl-1, 4-naphthoquinone. Thus all the vitamins now recognized except vitamin Bc were provided and the diet contained no crude vitamin carriers to supply any unrecognized essential nutrients. A second group of female rats was fed the above diet either with 5 per cent of a liver extract fraction or with 1 per cent of an eluate of a fuller's earth adsorbate of liver extract. When the female rats reached maturity, both groups were mated to males from the stock colony. In all, 1756 normal young were weaned from the females on the unsupplemented diet; 30 other weanlings had hydrocephalus. Of these 30, only 2 survived to the weaning age of 28 days. One died forty-six days after the hydrocephalic condition was detected. The other was still alive after one hundred and sixty days. No cases of hydrocephalus were observed in the 1020 young weaned by the females on the diet supplemented with the liver fractions. In addition no cases of hydrocephalus were observed among several thousand young reared by the females in the stock colony.

In a typical case of hydrocephalus, the head was observed to be dome-shaped and greatly enlarged. The amount of brain tissue was reduced and the affected region filled with serum. Gross diagnosis indicated that the hydrocephalus was internal. The diagnosis of doubtful cases was aided by observing the amount of light transmitted through the head when it was held between a light source and the eye of the observer. A normal brain transmitted little or no light but a hydrocephalic brain transmitted enough light to permit diagnosis of advanced cases which could not be recognized entirely by the shape of the head. This condition was first recognizable about the tenth day after birth. The authors suggested that some of the rats which died before 10 days of age might have been affected but not to such an extent as to be recognizable by the diagnostic methods used. The histologic examination of such rats probably would have added a great deal of fundamental data on the early stages in the development of the hydrocephalic lesions.

The liver fractions used supplied vitamin Bc and possibly additional, but as yet unrecognized, nutrients. No experiments were performed to test whether the addition of vitamin Bc in crystalline form to the otherwise unsupplemented basal ration would reduce the incidence of hydrocephalus. Three hydrocephalic rats were each given 500 mg. daily of liver eluate for four days and another 2 were each given 100 micrograms of crystalline Lactobacillus casei factor daily for four days. However all 5 rats failed to respond to the treatment. The failure of the 3 rats given liver eluate to respond to treatment indicated that the lesion was irreversible at the stage when treatment was begun.

It remains for further experiments to indicate what the active factor was in the liver concentrate which was responsible for the prevention of the hydrocephalic lesion. The incidence of hydrocephalus among the offspring of the females raised on the unsupplemented diet was only 1.7 per cent. Such a low incidence of any disease syndrome necessitates large and expensive experiments to produce data of statistical significance. When such an experiment involves fractionation of crude food sources to concentrate unknown essential factors, experimental procedure is further complicated. One item, not mentioned specifically by Richardson and Hogan, which strengthens their present paper and may aid in further studies is the definite difference in the number of young weaned per litter on the two diets. There was an average of 3.5 young in the 498 litters weaned by females maintained on the unsupplemented diet in contrast to an average of 5.8 young in the 175 litters raised by the females on the supplemented diet. The testing of vitamin Bc, in a prophylactic experiment similar to the liver supplementation, will be of interest to nutrition investigators.

The diet used in these experiments was a highly purified one. However the low incidence of the hydrocephalic syndrome may have been due to traces of the factor or factors responsible being carried by the components of the ration or due to sufficient intestinal synthesis and absorption to supply part of the maternal and fetal requirement. It would be interesting to determine if any alteration in the incidence or severity of the hydrocephalic lesion occurred in the next generation if the female survivors of the group born to mothers on the unsupplemented diet were maintained on the same diet throughout growth and reproduction. Likewise studies on the reduction of intestinal synthesis might produce an alteration in the occurrence and severity of the syndrome.

Most important is the demonstration again of the vital role of nutrition during the reproductive cycle upon the development of normal young during pregnancy and lactation. The likelihood of a diet consumed by human beings having a deficiency of these factor or factors appears to be remote. However some series of circumstances may produce excessive demands or a drastic deficiency for a limited period during the reproductive cycle.

THE ANTISTIFFNESS FACTOR FOR GUINEA PIGS

R. Wulzen and A. M. Bahrs (*Am. J. Physiol.* **133,** *P500 (1941)* described the existence in raw cream of an "antistiffness factor" for guinea pigs. The factor was absent from a diet of pasteurized milk, but present in raw whole milk. (The rations fed consisted of various types of milk or cream, and a standard addition was made to each type of milk, consisting of 10 per cent of skim milk powder, and adequate amounts of copper, iron, carotene, and orange juice.) On diets devoid of this factor a deficiency syndrome appeared, characterized first by a stiffness of the wrists which could be observed on attempting to manipulate the legs manually so as to overextend the wrists. In the continued absence of the factor, the animals died in an emaciated state after a month to a year without paralysis. An extract was made from raw cream which would cure the stiffness in a few days.

W. J. van Wagtendonk and R. Wulzen (*Arch. Biochem.* **1,** *373 (1942–43)*) found that a dose of 1 g. daily of raw cream cured the stiffness within five days and attempted to isolate the factor from raw cream. They described the preparation of a concentrate in the form of a yellow oil active in a dose of 0.1 microgram. When the same isolation

procedure was carried out on pasteurized cream, no active material was obtained. Data on the physiology of the deficiency were obtained by W. J. van Wagtendonk (*J. Biol. Chem.* **155,** *337* (*1944*)), who observed that it caused a derangement of phosphate metabolism, which in early stages of the deficiency was found to be a more constant sign than the wrist stiffness. The total inorganic phosphorus of the liver and kidney increased sharply in the deficient animals, but the most marked change was a decrease in the easily-hydrolyzable phosphorus. Control animals maintained a normal amount and distribution of the phosphorus fractions, and deficient guinea pigs when cured with the factor showed a return to the normal distribution.

W. J. van Wagtendonk and H. Lamfrom (*J. Biol. Chem.* **158,** *421* (*1945*)) further investigated the effect of the deficiency on phosphorus metabolism, in the muscles of guinea pigs kept as long as fifty-nine weeks on the deficient diet. The concentration of total inorganic phosphorus increased, and that of creatine phosphate and adenosine di- and triphosphate decreased after three weeks of the deficient diet. Changes in the distribution of the plasma protein during the deficiency were examined by W. J. van Wagtendonk and L. P. Zill (*Ibid.* **159,** *247* (*1945*)). Total plasma nitrogen increased during deprivation, due to a greater increase in globulin nitrogen than the decrease in albumin nitrogen. The values could be restored to normal by administration of the antistiffness factor, but only after prolonged periods.

W. J. van Wagtendonk and R. Wulzen (*J. Biol. Chem.* **164,** *597* (*1946*)) have described the isolation from sugar cane juice of the antistiffness factor in crystalline form. The method used was first to extract the cane juice at room temperature with ethyl ether in a semicontinuous extraction apparatus. This extraction yielded after solvent removal a dark green wax of potency 10^6 units per gram (1 unit was taken as the activity of 1 g. of raw cream, a dose which is curative in five days). This wax was dissolved in petroleum ether and extracted with 90 per cent methanol, which increased the potency by 50 per cent. Selective adsorption on magnesium oxide yielded a further purified orange-colored wax of 10×10^6 units per gram. High temperature molecular sublimation yielded an active fraction, and repetition of the process and crystallization from a 1:9 ethanol-benzene mixture, and repeated crystallization from petroleum ether, yielded a material of 500×10^6 units per gram. The product was pure white and melted sharply at 81.5 to 82°C. A dose of 0.002 microgram daily was sufficient to cure the wrist stiffness of deficient animals in one week and to raise the level of easily hydrolyzable phosphorus in the liver toward normal.

The authors reported that it was not known whether this compound is identical with that present in raw cream, but that work to establish its chemical structure is in progress. Nothing has been reported as to the possible significance of this factor in human nutrition. So far as is known other laboratories have not conducted experimental work with the "antistiffness" factor and hence the work just mentioned has neither been confirmed nor disputed.

DIETARY REQUIREMENTS AND GRANULOCYTE MAINTENANCE

The early experimental work which led to the discovery of what is now recognized as a deficiency of Lactobacillus casei factor (LCF) revealed the existence of anemia and granulocytopenia among the deficient animals of several species. L. Wills and H. S. Bilimoria (*Indian J. Med. Res.* **20,** *391* (*1932*)) found that deficient young

rhesus monkeys developed anemia and granulocytopenia, and the same phenomenon was observed independently by P. L. Day, W. C. Langston, and C. F. Shukers (*J. Nutrition* **9**, *637* (*1935*)). A. G. Hogan and E. M. Parrott (*J. Biol. Chem.* **132**, *507* (*1940*)) developed the deficiency in chickens and showed it to be characterized by failure of normal hematopoiesis, and F. S. Daft, L. L. Ashburn, and W. H. Sebrell (*Science* **96**, *321* (*1942*)) produced the same deficiency in rats fed simplified diets containing sulfonamides and showed that it led to leukopenia and sometimes to anemia.

The experiments of A. Kornberg, F. S. Daft, and W. H. Sebrell (*Arch. Biochem.* **8**, *431* (*1945*)) have shown that nutritional conditions other than the presence of LCF in the diet must be met to prevent blood dyscrasias in experimental rats. They found that granulocytopenia occurred in about half of a large group of rats fed a highly purified diet deficient in riboflavin. The blood dyscrasia was cured in only a quarter of the rats treated with riboflavin, but in 30 out of 33 treated with LCF. The granulocytopenia occurred also with high incidence among pair-fed control rats receiving riboflavin, and again could be cured or prevented by LCF.

Following up this indication that inanition favors the appearance of granulocytopenia, the same authors have studied the effects of low protein and protein free diets (*Science* **103**, *646* (*1946*)) and found that protein free diets caused the uniform development of granulocytopenia and anemia which were not cured by the addition of pure LCF alone to the diet, though LCF together with the addition of 18 per cent casein to the diet was highly effective in producing a cure. The nature of this requirement for protein for the prevention of granulocytopenia has been studied in detail by A. Kornberg (*J. Biol. Chem.* **164**, *203* (*1946*)).

The technic employed was to feed minerals and vitamins to young rats within a week after weaning on a protein free basal diet otherwise complete in energy sources. Eighty per cent of such rats developed granulocytopenia after an average of twenty-one days (range fourteen to forty-three days) on the basal diet. Total white blood cell counts, polymorphonuclear granulocyte counts, and hematocrit determinations were made at each examination, and granulocytosis was judged to be present when the polymorphonuclear granulocytes numbered 500 or less per cubic millimeter of blood. A severe anemia (hematocrit values less than 30 volumes per cent) usually appeared a few days after the onset of granulocytopenia, but in about 10 per cent of the rats anemia appeared first.

Each rat with granulocytopenia was used as a test animal as soon as the blood dyscrasia appeared. A test supplement was given to it for four days and the response was noted. Administration of 100 micrograms of synthetic LCF daily caused the average granulocyte count to rise from 316 to 936 cells per cubic millimeter on the average. The maximal response to this dose did not produce a higher level than 1800 cells per cubic millimeter in any rat among 42 which were given LCF alone. In tests to determine the influence of various proteins and amino acids on granulocyte production, 100 micrograms of LCF were administered daily for four days in addition to the nitrogenous test substance. The response of the rat to any treatment was regarded as "significant" if after the four day treatment the granulocyte count exceeded 200 cells per cubic millimeter. The nitrogenous test substances were incorporated in the basal protein free diet in place of an equal weight of dextrose. The proteins were given at an 18 per cent level in the diet, and for casein also at 30 and 50 per cent level. When the effect of amino acids in supplementing deficient proteins was tried, the total weight of the mixture of protein plus amino acids was 18 per cent of the diet.

The results, obtained with over 500 test rats, showed again that LCF alone or casein

alone failed to cure granulocytopenia, while a combination of LCF and either casein or egg white was effective. Oxidized casein, in which the methionine and tryptophane had been destroyed, could not replace untreated casein unless supplemented with these two amino acids. Dried human plasma was ineffective alone, but was fully supplemented by the addition of isoleucine. Zein and gelatin were ineffective; supplementation of the former with tryptophane, lysine, histidine, and valine, or of the latter with tryptophane failed to restore the granulocyte count. This result is not explicable in terms of our present knowledge and appears worthy of further experimental investigation.

A first test of an amino acid mixture containing all ten essential amino acids (i.e. essential for the maintenance of rapid growth) plus nine of the nonessential amino acids showed that when this mixture was fed at a level of 24.0 per cent of the diet (18.0 per cent of the free, natural acids), rapid increase in the number of granulocytes occurred. A test of the essential amino acids alone at a level of 13.25 per cent of the diet (8.7 per cent of the free natural acids) gave rapid increase of the granulocytes. The same mixture when supplied at 33.2 per cent of the diet (18.1 per cent of free natural acids) produced a significant, but less rapid, increase in the granulocyte count. The same mixture of acids less either arginine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophane, or valine showed that only arginine could be dispensed with, without rendering the mixture ineffective in restoring the granulocyte count to a high value. Without arginine, the count rose during the test period to 2254 per cubic millimeter (average of 11 rats) whereas with arginine and the mixture at a level of 8.7 per cent of free natural acids it rose to 4181 per cubic millimeter (average of 16 rats). It appears that, just as in the requirement for growth, granulocyte production can occur in the absence of arginine, but for the greatest rate of production this amino acid must be present in the diet.

The failure of some rats to survive the four day test period could not be accounted for by the failure to ingest sufficient food, as shown by the fact that among rats fed deficient amino acid mixtures the daily food intake of the rats which survived four days was no greater than that of those which succumbed. As might be expected, those rats in which effective granulocyte response occurred generally grew at a rate of 1 to 4 g. daily, whereas no weight gain occurred when the granulocyte responses were poor.

Under the experimental conditions chosen to study the granulocyte response, the rats rarely survived long enough to study the anemia, but preliminary results indicated that the same ten essential amino acids, supplied together, were "fairly effective" in replacing casein for erythrocyte formation. This result compares with that of F. S. Robscheit-Robbins, L. L. Miller, and G. H. Whipple (*J. Exp. Med.* **77**, *375 (1943)*) who found that in dogs hemoglobin formation was favored by a mixture of the essential amino acids together with glycine.

HISTAMINE

Inasmuch as the pharmacologic effects of histamine are so striking, this substance is usually considered as a drug and it is often forgot that histamine is actually a metabolic product derived from the amino acid histidine. This former viewpoint was fostered by the fact that histamine was first isolated from a natural source as the result of the action of putrefactive organisms which had acted upon the amino acid histidine (D. Ackermann, *Z. f. physiol. Chem.* **65**, *504 (1910)*). Hence, when the material

was isolated from animal products by G. Barger and H. H. Dale (*J. Physiol.* **41**, *499 (1911)*) it was considered by most workers as an artifact which was present due to action of putrefactive intestinal bacteria. Barger and Dale (*loc. cit.*) were careful to point out, however, that histamine probably should be considered as a normal product of intestinal mucosa because they isolated it from fresh intestines. Many workers have reported the isolation of histamine from a great variety of tissues which have been carefully handled to prevent any possible contamination or bacterial action. Therefore, there seems little doubt that histamine is normally present in animal tissues and that its presence is not due to post mortem bacterial action. It is widely held that histamine arises from histidine by decarboxylation (M. Guggenheim,"Die biogenen Amine," *Third Edition, p. 361, Basel (1940)*).

Histamine is destroyed by an enzyme known as histaminase (C. H. Best and E. W. McHenry, *J. Physiol.* **70**, *349 (1930)*). Histamine does not normally appear in appreciable quantities in the urine. However, it is claimed that in severe toxemias of pregnancy an increased quantity of histamine is excreted in the urine (*Nutrition Reviews* **1**, *134 (1943)*).

The physiologic role of histamine which occurs normally in tissues is much less clear than the pharmacologic effects of injected histamine. It has long been apparent that histamine is closely related to a gastric secretogogue which may be obtained from gastric mucosa (F. C. Koch, A. B. Luckhardt, and R. W. Keeton, *Am. J. Physiol.* **52**, *508 (1920)*). J. Sacks, A. C. Ivy, J. P. Burgess, and J. E. Vandolah (*Ibid.* **101**, *331 (1932)*) isolated histamine from extracts prepared from pyloric mucosa. It was then frequently assumed that histamine was identical with the substance known as gastrin. Reasons for doubting this assumption have been summarized (M. I. Grossman and A. C. Ivy, *Gastroenterology* **7**, *134 (1946)*). Certain allergic phenomena, asthma and sensitivity reactions, may be reproduced experimentally by the administration of histamine. The evidence cited by S. Rothman (*Physiol. Rev.* **21**, *357 (1941)*) indicates that the skin reactions to hypersensitivity are mediated by the liberation of histamine or some similar substance ("H-Substance"). Further support of this point of view has come about through the development of antihistamine substances (R. O. Roblin, *Chem. Rev.* **38**, *255 (1946)*) which relieve certain of the hypersensitivity reactions, particularly the skin reactions.

From the nutritional standpoint, histamine is of particular interest because of an investigation by E. Åkerblom (*Skand. Arch. Physiol. (Suppl.)* **68**, *1 (1934)*) of the disease known as "Futterrehe" or "founders" which occurs in horses after eating large quantities of grain, particularly rye. This disease of horses is characterized by lesions of the hoof, fever, muscle tremors, loss of appetite, a weak, rapid pulse, an increased respiratory rate, abdominal distention and signs of colic, polyarthritis, and frequently death. The symptoms of the disease could be reproduced by simultaneous administration of 10 g. of histidine and a culture of B. coli or by feeding the same microorganism plus hemoglobin, which is a rich source of histidine. It is well known that B. coli can decarboxylate histidine to histamine. Histamine could be isolated from the gastrointestinal contents of the animals dying of the disease. The symptoms of the disease could further be reproduced by intravenous or subcutaneous administration of histamine itself. It was concluded that this disease is due to the absorption of histamine from the gastrointestinal tract of the afflicted animals, the histamine arising from the histidine of the ingested grain protein. The decarboxylation of the amino acid depended upon the presence of a strain of colon bacillus which could produce this transformation. Such

strains were found sporadically in the stools of the horses.

As mentioned above, histamine in small quantities is widely distributed in animal tissues. Thus, it has been isolated from blood, traces from urine, bile, from the skin, liver, spleen, and various muscles. In M. Guggenheim's summary (*loc. cit.*) of the distribution of histamine in animal tissues, two of the richest sources are skin and lung tissues. F. Alexander (*Quart. J. Exp. Physiol.* **33,** *71 (1944)*) has investigated the distribution and metabolism of histamine in mice. The estimation of the amine was carried out by determining the effect of an extract of the tissue on guinea pig's intestines and cat's blood pressure. Each assay was checked by the two methods. Normal mice were killed by a blow on the head and divided into three parts: the entire skin, the complete alimentary tract, and the remainder of the body. The histamine content of each of these three fractions was then determined. The histamine content of the skin of these normal mice ranged from 60.0 to 72.0 per cent of the total histamine content of the animal. This observation is of particular interest because of the role of histamine or a histamine-like substance in sensitivity reactions in the skin. A second series of similar mice was injected intravenously with 3.0 mg. of histamine. These latter animals were kept in metabolism cages before they were killed at intervals after the injection. The collected excreta were assayed for histamine. The additional injected histamine administered to this group of mice was concentrated in tissues other than the skin and gut. After twenty-four hours there was little, if any, of the additional histamine to be found in any portion of the body of the animal. However, the histamine content of the excreta accounted for only some 40 per cent of the administered dose of 3.0 mg. The major portion of the excreted base, 37.7 per cent of total dose, was recovered from the urine. It was apparent, therefore, that some 60 per cent of the histamine which had been administered was destroyed within the body of the animal. Alexander points out that in these experiments on mice the urine contained histamine only when there was a very large excess in the body. He further observes that if the appearance of histamine in the urine in certain pathologic conditions in man can be taken to reflect large excesses in the body, the body excess must be enormous. It seems probable that if histamine is greatly increased during the toxemia of pregnancy it is likely due to some derangement in the ability of the kidney to destroy this base. It is not known whether a derangement exists in toxemia resulting in the excessive formation of histamine.

BLOOD PROTEINS IN DIABETIC RETINOPATHY

It is seldom disputed that there exists a specific type of retinal disease associated with diabetes mellitus. It affects young diabetic patients as well as older ones and those with severe, as well as with mild, diabetes; it may appear in the absence of hypertension, albuminuria, or impaired renal function. Its incidence bears a direct relation to the duration of diabetes, but the etiology of this type of retinopathy remains unknown.

The pathologic changes in diabetic retinopathy are essentially due to hemorrhage from small capillaries and veins of the retina (S. Hanum, *Acta Ophth.* (*Suppl.*) **16,** *3 (1939)*). The macular area is characteristically involved earlier and more severely than the peripheral retina. Once diabetic retinopathy occurs, its resistance to known methods of treatment is generally recognized. The lesions may persist for years with little change, but more frequently they

progress in spite of treatment, occasionally until the patient has complete loss of vision. Some work has been done which suggests that improvement may follow meticulous care of the diabetes but, as a rule, since visual acuity seldom is decreased in the early stages of retinal disease, advanced changes may be present when the condition is discovered. For this reason, any attempt to increase knowledge of the pathogenesis and treatment of this distressing complication of diabetes mellitus should receive careful attention.

L. A. Lewis, R. W. Schneider, and E. P. McCullagh (*J. Clin. Endocrinology* **4**, *535 (1944)*) reported that the pattern of the levels of protein in the plasma is frequently abnormal in cases in which the diabetes mellitus has been poorly treated. Usually the total quantity of protein is normal or slightly reduced, but the level of albumin is reduced and the concentration of beta globulin is increased. As a rule, these abnormal levels can be restored to normal by adequate treatment of the diabetes providing complications, such as retinopathy, renal insufficiency, and the like, are not present.

A further report by these investigators (R. W. Schneider, L. A. Lewis, and E. P. McCullagh, *Am. J. Med. Sci.* **212**, *462 (1946)*) concerned a study to determine the possible correlation between alterations in the plasma proteins and retinal changes in 31 cases of diabetic retinopathy and the effect of high protein diets on the retinopathy and the diabetes. Although diabetes mellitus is distributed almost equally between the two sexes, 26 (84 per cent) of these 31 patients with retinopathy were women; 80 per cent of the 31 patients were between 40 and 70 years of age and the average duration of diabetes was eleven years. Diabetic retinopathy was not recognized until diabetes had been present for two and a half to twenty-five years. Hypertension was present in about half of the group and proteinuria was common when the diabetes was untreated but often disappeared during adequate treatment of the diabetes. The albumin, alpha, beta, and gamma globulin and fibrinogen of the plasma were estimated by the Longsworth modification of the Tiselius electrophoretic method and total protein values by Pregl's modification of the micro-Kjeldahl method for estimating total and nonprotein nitrogen.

Total plasma protein in a group of normal subjects ranged from 5.9 to 7.8 g. per 100 ml., and in 29 diabetic patients the value was within this range. The remaining 2 diabetic patients had severe renal disease. The normal range of the albumin fraction was considered to be from 3.7 to 5.1 g. per 100 ml. and the average was 4.1 g. In 28 (90 per cent) of the 31 cases of diabetes the level of plasma albumin was low and ranged from 2.06 to 3.8 g. per 100 ml. with an average of 3.2 g. The diabetes in 3 cases in which levels of albumin were normal had been under adequate management from three to nineteen months before the first test was made. In 25 normal subjects used as controls the beta globulin ranged from 0.65 to 1.07 g. per 100 ml. and the average was 0.81 g. In 29 (93 per cent) of the 31 cases the beta globulin fraction was elevated; the average was 1.29 g. per 100 ml.

Of these 31 patients 10 were selected for more detailed study because they were able to maintain excellent control of the diabetes throughout the study. In this group the intake of protein varied from time to time and the effects were correlated with shifts in the pattern of the levels of protein in the plasma. Electrophoretic analysis of the plasma protein of these subjects was made repeatedly.

Four of these 10 patients were given a low protein diet and one of the 4 maintained an intake of 50 g. of protein per day for ten months. In this one case the initial low level of albumin fell further. An intake of 80 g. of protein daily for three months by another patient elevated the level of albumin to nearly normal. In the remain-

ing 2 cases in which the patients received 77 and 84 g. of protein per day for ten days and five months respectively the levels of albumin were not altered.

The remaining 6 of these 10 selected patients were given much greater amounts of protein, chiefly in the form of meat, for periods ranging from three weeks to six months. In one case when the intake of protein was increased from 70 to 140 g. the level of albumin was normal at the end of three months. The same fraction rose in 2 others in three and six weeks when the intake of protein was increased from 80 and 100 g. to 120 and 140 g., respectively. In the fourth case it was necessary to put the patient on a diet of 136 g. of protein per day for six months before the level returned to normal. In the 2 remaining cases when the patients received 140 and 120 g. per day for three months and one year, respectively, the levels of albumin did not rise. Proteinuria persisted in each of these 2 cases.

As levels of plasma albumin became normal, beta globulin levels approached normal in most instances. In no instances did a fall in the level of plasma albumin occur following an increase of protein in the diet.

Of these 10 patients, the condition of 4 was regarded as improved as measured by changes in visual fields, disappearance of retinal hemorrhages, and improvement in vision.

Apparently in diabetes with retinopathy a great intake of protein is necessary for long periods to restore to normal the patterns of the levels of plasma protein. Improvement in the condition of the retina occurred only when the abnormal patterns of the levels of protein returned to normal. Although the need for study of a larger series of patients for a longer time is apparent, yet a trial of this form of suggested treatment as an adjunct in the prevention and treatment of diabetic retinopathy seems indicated.

HEAT PROCESSING AND THE AVAILABILITY OF LYSINE IN PROTEIN

Alteration of the physicochemical properties of proteins by heat is a characteristic of this group of foodstuffs. Whether the altered protein is more or less nutritious than the natural form depends upon the individual protein. Phaseolin, a protein of the navy bean, has greater nutritive value if cooked (C. O. Johns and A. J. Finks, *J. Biol. Chem.* **41**, *379* (*1920*)), whereas the exposure of cereals to dry heat results in a significant decrease in the biologic availability of the protein (A. F. Morgan, *Ibid.* **90**, *771* (*1931*)). The latter finding has been confirmed by studies on human subjects and on rats (*Nutrition Reviews* **2**, *167* (*1944*); **3**, *238* (*1945*); H. H. Mitchell and R. J. Block, *J. Biol. Chem.* **163**, *599* (*1946*)). Growth of young rats on diets containing heat-treated casein is improved by the addition of lysine (E. O. Greaves, A. F. Morgan, and M. K. Loveen, *J. Nutrition* **16**, *115* (*1938*)) and a similar result has been obtained with heat-treated human globin (H. B. Devlin and C. A. Zittle, *J. Biol. Chem.* **156**, *393* (*1944*)).

Observations by R. J. Block and co-workers (*Arch. Biochem.* **10**, *295* (*1946*)) re-emphasize the deleterious effect of dry heat on the availability of lysine in dietary protein and the resulting decrease in protein nutritive value of foodstuffs. Block *et al.* prepared a special "cake mix," rich in essential amino acids, for use in high protein diets for surgical patients. The composition of the mix in grams per kilogram was as follows: flour 510; sugar 197; dried egg white 115; lactalbumin 76; hydrogenated vegetable oil 52; dried yeast 30; molasses 11; and salt 9. This mix yielded a palatable toasted product, resembling commercial rusk

(Zwieback), if made into a batter with water, yeast, and flavoring and then baked. Toasting was accomplished by heating the baked cake in an oven at 130°C. for forty to sixty minutes. It contained 25 per cent of its calories in the form of mixed protein having a distribution of essential amino acids approximating that of whole egg protein.

The efficiency of the protein of the unheated and heated cake mix in the regeneration of tissues of adult rats, which had been made protein deficient by the feeding of a low protein diet (P. R. Cannon, E. M. Humphreys, R. W. Wissler, and L. E. Frazier, *J. Clin. Invest.* **23,** *601 (1944)*), was compared with that of an equal quantity of a 1:1 mixture of lactalbumin and casein. For this purpose the test materials were incorporated into otherwise adequate diets so that the protein level was 9 per cent. All diets were isocaloric. Protein efficiencies of the test diets, expressed as gain in weight per gram of ingested protein during a fourteen day period, were as follows: lactalbumin-casein mixture, 3.3 to 3.6; unheated cake mix, 3.3 to 3.5; baked cake mix, dried at room temperature, 2.4; baked cake mix, dried at 60°C., 0.6; baked cake mix, dried at 60°C., plus lysine (0.36 per cent), 2.7; toasted cake mix, 0 to 0.8; and, toasted cake mix plus lysine (0.63 per cent), 3.2. Although the data on the effect of additions of lysine are too meager to permit estimation of the extent of nonavailability of the lysine in the heated preparations, it is evident that a considerable portion must have been destroyed or altered. The evidence of impaired nutritive value of the protein in the toasted cake mix is particularly impressive.

Dry heating of protein is not necessarily associated with chemical destruction of lysine. Acid hydrolysates of heated and unheated casein yield the same quantities of lysine (R. J. Block, D. B. Jones, and C. E. F. Gersdorf, *J. Biol. Chem.* **105,** *667 (1934)*), and only tryptophane is required to restore the nutritive value of acid hydrolysates of heat-impaired protein (W. H. Seegers and H. A. Mattill, *Ibid.* **110,** *531 (1935)*). More recently, H. H. Mitchell and R. J. Block (*Ibid.* **163,** *599 (1946)*) have reported a definite impairment by heat of the digestibility and biologic value of the proteins in a cereal mixture with no evident chemical destruction of lysine. According to Block, and Cannon *et al.*, a possible explanation of the damaging effect of dry heat is the formation of peptide bonds which are resistant to the action of the digestive enzymes. They suggest that a linkage between the epsilon amino group of lysine and a free carboxyl group of dicarboxylic amino acids may yield an unnatural dipeptide which is not available as a source of free lysine. Studies of the free amino nitrogen of heat-treated protein and of the products of its enzymatic hydrolysis may throw light upon this interesting biochemical problem. Whatever the explanation may be, it is significant that, according to present evidence, the biologic nonavailability of the lysine of heat-treated protein would escape detection by the usual chemical and microbiologic methods which are based upon the analysis of protein hydrolysates.

The widespread use of heat-processed foodstuffs in American households makes it imperative that additional information concerning the factors responsible for loss of protein quality be acquired. Obviously, any processing procedure which destroys a specific nutrient is wasteful of food and should be avoided unless it is justified by other gains of nutritive importance. A full understanding of the limitations, as well as of the advantages, of foodstuffs in this category is a prerequisite for the accurate evaluation of dietaries and for the assurance of good nutrition.

STORAGE IRON

The metabolism of iron under various physiologic and pathologic conditions promises to be an exceedingly complex and interesting field of investigation—a field of paramount nutritional significance. The increasing potency and availability of radioactive iron have greatly enlarged the research possibilities as evidenced by the appearance of reports on tracer studies from a number of different laboratories.

The exceedingly low solubility of inorganic iron in the neutral pH range may be responsible for some of the well established peculiarities of iron physiology: (1) the low concentration of iron in blood serum (0.1 mg. per cent), (2) the nearly negligible excretion of iron by the kidney (less than a milligram per day in man), (3) the very small absorption of iron from the diet by well nourished animals. In fact, a special intestinal mechanism may be required (*Nutrition Reviews* **5**, *45* (*1947*)) to effect the greatly increased absorption of iron which occurs when iron is required by the organism (*Ibid.* **1**, *154* (*1943*)).

The insolubility of iron is also undoubtedly a factor to be taken into account when attention turns to the investigation of the exchanges among the various tissue iron fractions and the entry of dietary iron into these fractions. The iron of the body is present in three major divisions: circulating hemoglobin (55 to 60 per cent), muscle hemoglobin and iron-containing enzymes (10 per cent), and "iron stores" (30 to 35 per cent) (S. Granick, *Chem. Rev.* **38**, *379* (*1946*)). Of the iron stores about two-thirds, or 20 per cent, of the body iron is located in the spleen, liver, and bone marrow.

G. R. Greenberg and M. M. Wintrobe (*J. Biol. Chem.* **165**, *397* (*1946*)) have presented evidence that in normal animals, including man, only 10 or 15 per cent of the iron reserve (3 or 4 per cent of the total body iron) is drawn upon to build the red cells needed to replace those which continually "wear out." Each day, according to the most recent evidence, about 0.8 per cent of the red cells are broken down and replaced by new ones. If a small amount of radioactive iron is injected into the body, the new red cells presumably will be built with iron drawn from a mixture composed of (1) the injected radioiron, (2) the iron liberated from the old cells, and (3) whatever fraction of the storage iron comes into equilibrium with 1 and 2. The size of this fraction of the storage iron may be calculated by measuring the percentage of the tagged iron which appears in the circulating red cells, since the more the radioiron is diluted with storage iron, the less it will be used in the fabrication of new red cells, and vice versa. Greenberg and Wintrobe found when 5 mg. of radioiron (Fe^{59}) were injected into man, or proportionally less into smaller animals, that 10 to 15 per cent of the tracer dose was incorporated per day into the red cells. From this they calculated that a man with an estimated total iron reserve of about 1000 mg. has a "labile iron pool" of only about 130 mg. with which the estimated 25 mg. of iron from broken red cells is mixed and from which the equal amount of iron for new cells is withdrawn. Nearly all of the 5 mg. tracer dose entered the red cells within ten days which indicated little exchange between the labile pool and the rest of the iron stores. With large doses of iron, however, only a part of each dose appeared in the red cells; apparently, the rest escaped into the larger, more stable iron reserve.

D. H. Copp and D. M. Greenberg (*J. Biol. Chem.* **164**, *377*, *389* (*1946*)) have examined individual rat tissues following administration of radioiron and since they used iron dosages comparable with those of G. R. Greenberg and Wintrobe, it should be possible to localize the "labile iron pool" from their data. These investigators used newly available Fe^{55} instead of Fe^{59}. Fe^{55} can be prepared with very high radioactivity

since it is made by bombardment of manganese and hence, the amount of ordinary iron may be as small as desired. Almost half of injected iron (intraperitoneal or intravenous) was found during the first twenty-four hours in the liver, with much lesser amounts appearing in the spleen and bone marrow. The specific activity (the ratio of radioiron to total iron) was approximately eight times greater in the liver than in the spleen. Thus, the liver appears to contain a large portion of the "labile iron pool" whereas the spleen contains more of the stable iron reserves. The iron of the bone marrow, like that of the liver was relatively "labile," although quantitatively not so important.

The data, however, indicate that there is actually no homogeneous labile pool, since the mode of administration whether oral, subcutaneous, intraperitoneal, or intravenous, greatly influenced the relative deposition in the various tissues. This would not be expected if a truly tracer dose of iron were merely indicating the normal "turnover" or equilibrium exchange between the several tissues. Thus, the liver took up the greatest relative amount of the dose following intravenous injection, whereas with slower methods of administration, especially when the iron was given orally, the bone marrow and spleen took up larger relative amounts. Previous workers have shown that larger doses of injected iron are rapidly taken up by the liver (S. Granick and P. F. Hahn, *J. Biol. Chem.* **155**, *661 (1944)*). Apparently, even the small doses of iron injected by Copp and Greenberg (0.05 mg.) were sufficient to have increased the serum-iron level fivefold. With oral administration of iron there has been found to be relatively small increases in serum-iron concentrations. The amount of iron taken up by the liver may depend on the serum-iron level. From the difference in behavior of orally and intravenously administered iron it seems probable that part of the labile pool reported by Greenberg and Wintrobe is even smaller and more rapidly turned over than the rest of the iron fraction which they have described.

Copp and Greenberg have included in their report extensive observations on the absorption and distribution of radioiron in iron-depleted as well as normally nourished young and mature rats. Most of these observations are beyond the scope of the present review, but will merit careful study. In confirmation of results with dog and man, it was found that in iron deficiency, iron absorption from the gastrointestinal tract is greatly increased. Observations on the incorporation of radioiron into the red cells appear to be consistent with the idea that in the deficient animal the iron for new red cells is drawn from a much smaller iron pool than that in the normal animal—a reasonable concept.

In summary, there seems to be good evidence that the iron stores are not homogeneous in their behavior. It appears that only a fraction of the iron reserve comes into equilibrium with iron liberated from "worn out" red cells. It is, however, likely that even the labile pool, as measured by Greenberg and Wintrobe, is not homogeneous, but consists of tissue iron with a range of turnover rates. An analogy is perhaps permissible between the iron stores and the calcium of bone. Serum calcium exchanges with bone calcium at a multitude of different rates, depending on how close the calcium is to the surface of a bony spicule or how deep it is buried in solid cortical bone.

BERIBERI IN A JAPANESE PRISON CAMP

Reports describing the results of surveys of the nutritional status of former prisoners of war have been discussed (*Nutrition Reviews* **5**, *37 (1947)*). Observations made

by one of the victims during imprisonment proves to be an interesting supplement to these studies. R. E. Hibbs (*Ann. Int. Med.* **25,** *270* (*1946*)) spent thirty-four months with approximately 8000 Americans who had surrendered on Bataan and Corregidor. His study was handicapped by meager laboratory facilities and the lack of cooperation and supplies but, nevertheless, the account is a valuable and interesting one.

The diet which these men received varied from time to time. In total calories it amounted to between 900 and 2000. The main staple was rice which was of medium mill but at times corn, camodies, casaba, and gabi were substituted. The pericarp of the rice which contains vitamin B was about half removed in the milling of the grain. Vegetables, the leafy portions, fresh carabao meat, fish, oil, sugar, and small amounts of fresh fruit were also issued. In general, the diet was high in carbohydrate and low in fat content, thus increasing the requirement for thiamine.

A clinical picture resembling that of beriberi was the most common disorder among the prisoners, everyone in the camp having some form of the condition at one time or another. The disease was directly responsible for more deaths than any other vitamin deficiency disorder. The symptoms were comparatively late in appearance and came at the same time as symptoms of vitamin A deficiency. In the cases of dry beriberi, painful feet or hands were predominant complaints. Motor disturbances were mild except in a few patients who developed foot or wrist drop. Peroneal or radial nerve paresis or paralysis were encountered but these developed suddenly without going through a painful stage. Only rarely did the patient progress from a painful state to motor paralysis. Whereas over 75 per cent had predominantly sensory disturbances or painful feet, only 2 per cent in the entire camp's history developed motor paralysis. In a few patients intraocular optic neuritis with gradual diminution of vision and optic atrophy was observed and a skin lesion similar to erythema nodosum which was given the name "beriberi spots" was encountered.

The first symptoms of the peripheral neuritis were stiffness, heaviness, or a tired feeling in the arches of the feet. This was followed by aching in the arch and soles of the feet which progressed to a dull, throbbing, deep bone ache in the whole foot. Soon sharp shooting pain appeared radiating from the arch to the tip of the toes. Burning pain, especially on the soles of the feet, paresthesias, and extreme tenderness developed next. These symptoms increased in severity until the patient was "half crazy." There was no relief. He would rub his feet or just look at them and cry.

Along with these local symptoms there was loss of sleep, nervousness, loss of weight, fatigability, palpitation, shortness of breath on exertion, and emotional instability. Accompanying these were gastrointestinal symptoms such as distention, excessive gas, foul breath, and aerophagia. On examination, the patients were thin, emaciated, very nervous, and obviously suffering from acute pain. The affected parts revealed mottled, erythematous skin. The soles of the feet and palms of the hands were often fiery red. Excessive sweating was present and pronounced dermatographia indicated vasomotor disturbance. There was a stocking and glove distribution of the sensory disturbance usually to the level of the mid-forearm and mid-thigh. Deep and superficial tenderness was severe. Muscle strength, however, was affected very little and all patients were able to stand from a squatting position. The gait was so unique that it was called the Cabanatuan shuffle. It was protective in character. The weight was placed on the outer side of the foot, each step was slow and deliberate, the patient looked carefully for a smooth place, then gingerly placed his foot down and flung his arms upward.

Cardiovascular manifestations were en-

countered in the majority of the patients with beriberi. A diagnosis of beriberi heart disease was made when signs and symptoms of peripheral neuritis existed and symptoms referable to the heart were encountered for which there was no other demonstrable cause. Manifestations of beriberi heart disease did not necessarily occur in the severest cases and usually no edema was present. The symptoms varied from dyspnea, palpitation, irregularities of the heart beat, and sudden attacks of pounding of the heart to the symptoms of congestive heart failure with orthopnea and prostration. Seldom was there complaint of precordial distress. Roentgenograms were taken in 100 instances and all of these failed to show cardiac enlargement. The heart was usually hyperactive and the rate was usually 130 to 140.

In addition to this acute form, a more chronic variety with enlargement of the cardiac shadow usually of the right side and with cyanosis, enlargement of the liver, rales in the bases of the lungs, dyspnea, dependent edema, and a fast, thready pulse was encountered. Only 20 to 25 such cases were observed. Most of these individuals died and one who was given 10 mg. of thiamine chloride every day died despite such treatment. Consequently, it was difficult to be certain that this represented thiamine deficiency. A third form of beriberi heart disease manifested itself in acute dilatation of the heart, pulmonary edema, and death within a few minutes to twenty-four to forty-eight hours. These patients had peripheral neuritis and usually no edema. Occasionally they had symptoms referable to the heart such as palpitation or poor exercise tolerance. One minute prior to the onset they were walking around and the next minute they collapsed. Approximately 50 men died with such symptoms.

It is interesting to note how closely the symptoms of the first and third forms of beriberi heart disease observed by Hibbs corresponded to those described in swine by M. M. Wintrobe and associates (*Bull. Johns Hopkins Hosp.* **71,** *141 (1942)*). In the experimental animals, a deficiency of thiamine was associated with loss of appetite, some impairment of growth, and acute symptoms of cardiac failure. Not infrequently, sudden death would occur. Marked impairment in the function of the heart was also manifested by striking irregularities of the heart (M. M. Wintrobe, R. Alcayaga, S. Humphreys, and R. H. Follis, *Ibid.* **73,** *169 (1943)*).

Although Hibbs made the common assumption that the peripheral neuritis observed in his subjects was, like the cardiac manifestations, caused by thiamine deficiency, it is noteworthy that, in a group of 96 men representing the severest cases of painful feet, improvement following treatment was greatest in those who were given brewers' yeast, liver extract, and a vitamin B-complex preparation in addition to 20 mg. of thiamine chloride subcutaneously. The patients receiving only thiamine chloride showed no more improvement of the pain and tenderness in the extremities than the control group who received placebos, although in these men, as in those given vitamin B-complex in addition to thiamine, appetite improved, insomnia and irritability decreased, emotional instability improved, the pulse rate dropped, and exercise tolerance improved. Dyspnea, palpitation, and arrhythmia of the heart were other findings which showed early improvement.

In the face of such observations it is difficult to understand why clinicians persist in assuming that thiamine deficiency rather than the lack of some other member or members of the vitamin B-complex is the cause of the peripheral neuritis encountered in the complex vitamin deficiency syndrome, beriberi. As M. M. Wintrobe has shown (*Tr. Assn. Am. Phys.* **57,** *55 (1942)*) the evidence that thiamine is "the anti-neurotic vitamin" is weak and needs re-evaluation. The same author has pointed out (*Arch. Int. Med.* **76,** *341 (1945)*) that, on the other hand, the role of thiamine deficiency in producing cardiac dysfunction is well established.

MATERNAL VITAMIN A INTAKE AND OCULAR ABNORMALITIES IN THE OFFSPRING

J. Warkany and E. Schraffenberger (*Proc. Soc. Exp. Biol. Med.* **57,** *49 (1944)*; *Arch. Ophth.* **35,** *150 (1946)*) have shown that the eyes of offspring born of vitamin A deficient mothers may show any of the following abnormalities: absence of anterior chamber, iris, and ciliary bodies; failure of vitreous formation, with the development in its place of connective tissue; eversion of the retina, and retinal disorganization. In some instances the defects consisted in the development of a mass of connective tissue behind the lens of the eye.

B. Jackson and V. E. Kinsey (*Am. J. Ophth.* **29,** *1234 (1946)*) have repeated these experiments, seeking to determine particularly the degree of deficiency in the mothers which will lead to ocular maldevelopment. They used weanling female rats which were grown to maturity without the accumulation of hepatic stores of vitamin A. This was done by placing them on a basal vitamin A deficient preparatory diet in accordance with the procedure described by Warkany. The diet consisted of ground whole wheat, 74; crude casein (Borden), 15; brewers' yeast (Mead Johnson), 10; and sodium chloride, 1. This was supplemented by vitamin D and carotene sufficient to allow growth and sexual maturation but insufficient to allow the accumulation of significant hepatic stores. When the females were 90 to 100 days old, they were fed a purified diet deficient in vitamin A and were given only minimal amounts of vitamin A during pregnancy. The vitamin A content of the blood was measured by the micromethod developed by O. H. Lowry and O. A. Bessey (*Federation Proceedings* **4,** *268 (1945)*).

The first experiment, carried out in 57 female rats and designed simply to reproduce the earlier experiments, confirmed them entirely. Only 4 of the mothers carried their fetuses to advanced stages of development. The eyes of the young of these litters showed the abnormalities which were described by Warkany and Schraffenberger.

To determine the limiting level of vitamin A dosage in female rats producing young with abnormal eyes, similarly prepared female rats were given 2, 3, 5, or 10 I.U. of vitamin A daily. They were then bred and the supplement was continued at the same level. Twenty out of 30 animals became pregnant but 5 of the pregnant animals died within three weeks after mating. At the time of death, 2 of these had completely resorbed their fetuses. Five had fetuses in various stages of resorption. Young obtained from mothers fed less than 3 to 5 I.U. daily showed the eye defects already mentioned whereas all of those born of mothers receiving more than this amount of the vitamin had normal eyes, even though the vitamin A dosage was insufficient to allow normal delivery in many cases. Furthermore, the degree of abnormality appeared to be related inversely to daily intake so that at the 5 I.U. level, except for one case, only abnormal retinal folding was observed.

Serum vitamin A values taken during pregnancy from female rats producing young having abnormal eyes were found to be less than 12 I.U. per 100 ml. in every instance (normal 75 I.U. per 100 ml.). Fetuses with normal eyes were obtained when the maternal serum vitamin A level was only slightly greater than this. Thus ocular defects occurred in the young of rats having serum levels approximately one-tenth or less of the normal. For ocular defects to appear the maternal vitamin A deficiency must be extreme, so advanced, in fact, that fetal resorption is common and normal birth is impossible.

It is of interest that marked folding of the retina (or inner layer of the optic

cup) is one of the most striking features observed in the abnormal eyes. In some cases this reduced considerably the space which in normal eyes is occupied by the vitreous body. This change was the first abnormality encountered as animals with increasing degrees of vitamin A deficiency were examined.

This study was undertaken also with the object of determining whether vitamin A deficiency in human mothers might be a cause of retrolental fibroplasia, a disease which results in blindness in almost 8 per cent of infants weighing less than 5 pounds at birth. The ocular defects which cause blindness in these infants closely resemble those found in the experimental animals born of vitamin A deficient mothers. To the extent that the physiologic processes associated with reproduction in human beings parallel those in the rat, it is inferred, from the fact that the ocular abnormalities occurred only when the vitamin A deficiency was extreme, that lack of this vitamin in human mothers is not a probable cause of retrolental fibroplasia.

INFANTILE SCURVY

A short report by I. Dogramaci (*New Engl. J. Med.* **235,** *185 (1946)*) reminds us that scurvy in infants has not disappeared. This short paper records 241 cases of scurvy seen in the Infant's and Children's Hospitals in St. Louis during the last ten years. The criteria used in selecting the cases were not strict. However, it is surprising to note such a large group of cases occurring in these clinics. In most of the cases, the first symptoms appeared between the ages of 5 and 11 months. The seasonal distribution indicated a greater incidence of scurvy in these clinics during July and August and the lowest incidence during October, November, and December.

The most usual first signs or symptoms as recorded in these 241 cases were tenderness in one or both lower or upper extremities, irritability and generalized tenderness, and anorexia and failure to gain weight. This was the order of frequency of these symptoms. The most frequent signs noted on the first physical examination were tenderness in one or both lower or upper extremities, enlarged costochondral junctions, involvement of gums, and swelling of one or both lower or upper extremities, and petechiae or ecchymoses. A hypochromic anemia was found in 12 per cent of all the patients. Other accompanying diseases were infections of the upper respiratory tract, other infectious diseases, and rickets.

A tabulation is presented of some explanations for the reasons for inadequate intake of ascorbic acid as given in the records of 172 of these cases. It is of interest that most of these factors enumerated had to do with the failure of the children to take specific antiscorbutics. In 7 of the cases, however, the informant insisted that the child had been receiving adequate quantities of orange juice or synthetic ascorbic acid. It would have been of considerable interest if other data had been presented on these 7 cases. It is of interest that most of the babies had been under the care of a physician and that the mothers seemingly were aware of the necessity of ascorbic acid in the children's diets. However, either because the child had refused orange juice, or because the particular source of ascorbic acid had produced symptoms in the child, the infant was permitted to develop scurvy. Although Dogramaci does not report the detailed supplementary feedings of these children, it is apparent that the diets contained few, if any, freshly prepared fruits and vegetables in addition to milk. The type of milk given these children is

not indicated. It is well, however, to recall in this connection A. F. Hess's statement ("Scurvy, Past and Present," *J. B. Lippincott Co., Philadelphia, p. 35 (1920)*) to the effect that "It is true that in the literature we meet with scattered reports of scurvy in breast-fed babies and that these cases seem to constitute a noteworthy group; in point of fact, they are comparatively few." It appears, therefore, that the first step in the building of a sound antiscorbutic diet for children is breast-feeding. The further insistence by the physician on the early inclusion of other antiscorbutic foodstuffs, such as orange juice, tomato juice, and other freshly prepared fruits and vegetables, should prevent all cases of this disease except in the rare instance where the parent or guardian refuses to assume responsibility for the proper feeding of the child.

PROTEIN AND PHYTIC ACID IN SEED MEALS

For several decades it has been known that phytin, which is the mixed salt of potassium, calcium, and magnesium with phytic acid (inositol hexaphosphoric acid) is the principal form of phosphorus and inositol in seeds. Phytin and phytic acid were long thought to occur only in the vegetable kingdom, but in recent years they have been found to occur also in soils and in the red blood cells of some animals. The importance of phytic acid and phytin in nutrition, particularly in the effects they have upon the absorption of iron (*Nutrition Reviews* **2,** *45 (1944)*) and calcium (*Ibid.* **3,** *61 (1945)*) from the intestine is well established.

T. D. Fontaine, W. A. Pons, and G. W. Irving (*J. Biol. Chem.* **164,** *487 (1946)*) have reported a study of the relationship between phytic acid and the proteins of peanuts and cottonseed. An earlier paper of T. D. Fontaine, G. W. Irving, and K. S. Markley (*Ind. Eng. Chem. (in press)*) had reported that these meals contain a dialyzable substance which exerts a strong effect on the solubility characteristics of the meal proteins. This dialyzable substance has now been identified as phytin, and the protein-phytic acid solubility relationship has been determined over a wide pH range for peanut and cottonseed meals as well as for the corresponding dialyzed meals and isolated proteins. The properties of the phytases present in the seed meals were also investigated, and a limited number of observations on the protein-phytic acid relationship in soybean meal were obtained.

The authors discuss at some length the theoretical and practical significance of the protein-phytic acid relationship which they have observed, supporting their arguments with data obtained on other seed meals and previously reported in the literature. These other reports cover oat flour, horse-bean meal, hemp meal, and wheat flour. From solubility curves such as those obtained in the present investigation it is possible to predict the amount of phosphorus present as impurity in isolated protein preparations if the pH of the solution used to extract the crude meals, and also the pH of precipitation of the protein are known. Alternatively, the amount of phytic acid present as impurity could be predicted.

It is also possible to use the solubility curves to deduce the best conditions for solution and precipitation of the meals so as to obtain the minimum contamination of the protein by phytic acid. This is an important application to the purification of seed proteins because phytic acid is a potential major impurity in seed proteins.

The results also indicate that the possibility, sometimes considered in the past,

of using the mother liquor remaining after the precipitation of proteins from meals such as cottonseed meal for the isolation of phytin, cannot be economically or commercially feasible. However, by leaching the crude peanut, cottonseed, or soybean meals with dilute acid at pH 5.5, 5.75, or 5.0 respectively, it should be possible to dissolve 80, 85, and 55 per cent respectively of the phytic acid which they contain, free from any appreciable amount of protein nitrogen. The leachings could then be used for phytin preparation, and the leached meals for protein isolation.

The possibility is broached that proteins obtained in this way from leached meals having low phytic acid content might have qualities so superior for industrial use to render worth while this additional step in the isolation procedure.

THYROPROTEIN IN CATTLE FEEDING

Supplementation of animal feeds with thyroprotein, as a means of obtaining higher production, continues to receive study. The practice has been recommended for chickens during low egg production seasons (*Nutrition Reviews* **4,** *297* (*1946*)), and the present review summarizes large scale experiments in the feeding of thyroprotein to cattle.

Preliminary studies were carried out by E. P. Reineke and C. W. Turner in this country (*J. Dairy Sci.* **25,** *393* (*1942*)) and by K. L. Blaxter in England (*J. Endocrinology* **4,** *237*, *266* (*1945*)). They found that milk production by cows could be increased significantly by feeding iodinated casein during the middle and latter portion of lactation. A 20 per cent increase in milk yield was considered optimal from the standpoint of prevention of hyperthyroidism.

A large scale test was then carried out by K. L. Blaxter (*J. Agr. Sci.* **36,** *117* (*1946*)). It embraced ninety-seven farms throughout most of England and Wales. One thousand, one hundred and sixty-four cows were selected for the study and 1008 cows were finally included in the report. Six breeds of cattle were included, as well as a number of crossbreed animals. Cows were paired as nearly as possible on the basis of age, stage of lactation, and milk yield. Each participating farm furnished three to fourteen pairs of cows, with six being the most common number. One cow from each pair was maintained on the stock ration which was being fed to that herd, and the other was given a supplement of 4 pounds of feed containing 20 g. of iodinated casein, fed as a compressed cube. Although the thyroxine or iodine content of the casein was not given, the 20 g. level was found in a preliminary test to produce approximately a 20 per cent increase in milk yield.

The experiments were divided into three periods: first, a two week period during which both the control and experimental cows were fed and managed in the usual way; second, an experimental period of six weeks, in which the experimental cows received the iodinated casein cube; third, a final period of four weeks, in which both cows in each pair were again treated alike. Many data were assembled, including records of milk yields and fat contents, feeding and health records, reports on the heart beat rates of the cows, their weights, and general condition.

The extra food allowance for each experimental cow was not duplicated among the control animals, so that additional milk yields might be expected on this basis alone. This point was recognized by Blaxter, but data were presented to show that in well fed herds the increase due to feeding the cubes (placebos) is 1 pound per day or less. Moreover, the experimental animals were found to require the extra feed

to prevent excessive weight loss. Even with the supplement, 30 treated cows on four farms lost 10 pounds per animal during the experiment while the control animals gained 8 pounds. The differences were greatest for light breeds, where losses on the experimental diet averaged 48 pounds per cow.

The mean increase in milk production, during the third and fourth weeks of the treatment of five hundred and four pairs of cows, was 5.54 pounds per day. The mean yield at the start of the experiment was 25.0 pounds per day, so that the per cent increase was 22.2. When responses were calculated at the twentieth week of lactation for cows giving 25 pounds daily, the increases were largest for the small breeds, ranging from 6.84 pounds per Ayrshire cow down to 5.22 pounds for each Friesian cow. This observation, together with the weight losses listed above, was interpreted to indicate that 20 g. of iodinated casein was too large a dose for the smaller cattle. It was further stated that on one farm where the cows were mistakenly fed up to 60 g. of casein per day, the daily increase in milk production reached 13.5 pounds within two weeks, but at the same time severe symptoms of thyrotoxicosis were observed. When the dosages were reduced the symptoms disappeared, and the cows apparently returned to good health. Further evidence suggesting that the 20 g. dose might lie near the harmful range was the fact that heart beat rates were elevated in the experimental cattle, on some farms as much as 20 per minute. Nervousness and digestive disorders also appeared to be more prevalent than in control animals.

A comparison of cows at different ages and stages of lactation revealed that the increases in milk yield were smallest among heifers (4.5 pounds per day compared with 6.9 pounds per day among old cows). This was apparently due to the higher metabolic rate of the younger animals, since their normal heart beat rates were higher. Similarly, the casein was markedly superior in mid-to-late lactation. Cows yielding 20 pounds of milk per day after ten weeks lactation increased 3.9 pounds per day while on the treatment, whereas cows giving 20 pounds daily after thirty weeks lactation increased 7.8 pounds. These and other data also indicated that the stimulation was greatest among the heavy milkers.

The final phase of the experiment was ended abruptly with some of the cattle, after thirty-nine days of treatment, while others received a gradually reduced supplement during the twenty-ninth to the forty-ninth day. Both groups thus received essentially the same amount of thyroprotein. The gradual reduction was accompanied by a similar gradual drop in milk yield, as contrasted with an abrupt drop in the other group, and was considered to be a more satisfactory method for terminating the treatment.

The scope of the present study and the mass of data obtained are impressive, and the results probably represent the best that can be obtained in field studies of this type. It is perhaps unfortunate that the feeding of the casein cubes and the keeping of daily records had to be left to the individual farmers, for mistakes were encountered and large variations sometimes occurred (as in butterfat determinations) which rendered the conclusions of little significance.

From the results of the survey, the author suggested that iodinated casein could be profitably fed to selected cows during the late winter months when production is usually heaviest. A 15 g. dose is recommended, especially for light weight breeds, and the author feels that no permanent damage would be done to the cows under these conditions.

CITRIC ACID SYNTHESIS IN HUMAN SUBJECTS

Citric acid synthesis has been demonstrated in the animal body by C. C. Sherman, L. B. Mendel, and A. H. Smith (*J. Biol. Chem.* **113,** *247* (*1936*)). Similar observations in human subjects have been found by J. E. Strom and M. L. Hathaway (*J. Nutrition* **32,** *327* (*1946*)). These workers compared the urinary citric acid excretion of 3 subjects fed a purified diet containing no citrates with the citric acid excretion of the same subjects when fed a natural-food diet which supplied an average of 197 mg. of citric acid per day. Since C. C. Sherman, L. B. Mendel, and A. H. Smith (*J. Biol. Chem.* **113,** *265* (*1936*)) had shown that animals excreted small and relatively constant amounts of citric acid in their feces, no fecal analyses for citrates were made in the present experiments.

When the subjects were fed the purified diet for a period of forty-five days, the daily citric acid excretion was observed to vary from 563 to 1179 mg. for subject A, from 673 to 994 mg. for subject B, and from 437 to 1019 mg. for subject C. When the natural-food diet was fed for a period of twenty-one days, the daily citric acid excretion of the 3 subjects was found to vary within the same range observed when the purified diet was fed. The citrates ingested in the natural-food diet had not influenced the citric acid excretion. Individual variations in daily citric acid excretion of the same magnitude were observed on the two diets. During the forty-five day period of maintenance on the citrate-free, purified diet, the subjects excreted a total of 38, 39, and 34 g. of citric acid, respectively. The authors calculated that at the beginning of the experiment the subjects had citric acid stores in their skeletal structures of roughly 43, 45, and 47 g., respectively. No decline was observed in citric acid excretion toward the end of the forty-five day period even though the amounts of citric acid excreted were equivalent to 70 to 90 per cent of the calculated citric acid stores. In addition no increase in calcium excretion was observed during the entire experimental period. Since citric acid and calcium are believed to be intimately associated in bone, these data are strong evidence that citric acid synthesis does occur in human tissues when a ration containing no citrates is fed.

An interesting relationship was observed between the citrate excretion and the phase of the menstrual cycle. In each subject, the highest excretions occurred during the mid-period of the cycle and the lowest excretions during menstruation. This represents confirming evidence for the relation of urinary citric acid excretion to the menstrual cycle and the steroidal reproduction hormones described by E. Shorr, A. R. Bernheim, and H. Taussky (*Science* **95,** *606* (*1942*)).

NOTES

Letter to the Editor

Dear Sir:

In the December number of Nutrition Reviews, the interesting review on thiamine contains a paragraph regarding fat synthesis which states: "... the failure of thiamine deficient rats to synthesize and deposit fatty acids is due to a diminished food intake." Reference is made to the paper by G. E. Boxer and DeW. Stetten, which was reviewed by your publication in October, 1944.

The recent review gives an inference which was specifically stated in October, 1944, that no attention was paid to the effect of thiamine upon food consumption prior to the work of Boxer and Stetten. In this sense, both reviews are misleading. I call attention to this because the readers of Nutrition Reviews may be confused by the failure to consider adequately the evidence regarding the relation of thiamine to fat synthesis. In particular, reference should be made to the following:

1. The first publication on the effect of thiamine upon fat synthesis was by G. H. Whipple and C. F. Church (*J. Biol. Chem.* **114,** *cvii* (*1936*)). Those workers used isocaloric feeding and clearly said so. In the first paper published from our laboratory (*J. Physiol.* **89,** *287* (*1937*)), results were reported for two experiments in which isocaloric feeding was employed; in one case 25 rats were used in each group. Definite reference was made to the failure of increased food consumption to account for the results in several subsequent papers from our laboratory (*J. Biol. Chem.* **125,** *653* (*1938*); *Ibid.* **128,** *45* (*1939*)). Results reported by Whipple and Church and all of the work reported from our laboratory are in agreement and show that thiamine has an affect upon fat synthesis, separate from the indirect action upon food consumption. The fact that isocaloric feeding was used in the early observations was ignored by Boxer and Stetten and in your reviews.

2. It could be claimed that the more recent work of Boxer and Stetten completely wipes out other work, as is assumed in the current and previous reviews. The conclusions of Boxer and Stetten should be considered critically. Examination of their paper indicates the following observations:

a. The number of animals in each group (5) was small.

b. Results for the two paired groups are misleading because there was a marked difference in the average initial weights of the two groups. The pair group given thiamine was 10 to 11 g. lighter at the start. If the two groups had had the same initial weight, it is likely that final weights and the final amounts of body fat would have been much different.

c. The proportions of deuterium in the body fat of the paired animals given thiamine were about the same as in the ad libitum group receiving thiamine, and were much greater than in the deficient group. This indicates that considerable synthesis took place in the isocalorically fed rats receiving thiamine.

3. It is difficult to understand how fat could be synthesized without the degradation of carbohydrate, in which thiamine is essential.

The weight of experimental evidence still seems to the writer to favor the assumption that thiamine is necessary for the synthesis of fat from carbohydrate and from protein.

E. W. McHenry
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Carious Lesions of the Enamel

J. Nuckolls and H. E. Frisbie (*J. Am. Coll. Dentists* **13,** *84* (*1946*)) have discussed the historical and present theories of the fundamental pathology of carious lesions in the enamel. Their comparative evaluation of the two theories which presently are given

credence by research workers in this field will interest all who are concerned with the relations of nutrition to the control of dental caries. W. D. Miller's theory was based on experimental studies which he interpreted as evidence that bacterial fermentation of carbohydrates resulted in acid formation and that the initial lesion of dental caries consisted of a decalcification of enamel and dentin ("The Microorganisms of the Human Mouth," *S. S. White Dental Mfg. Co., Philadelphia (1890)*). This theory has been elaborated upon by more recent workers who belong to this school of thought. The primary assumption of this group is that enamel is essentially nothing more than an inorganic complex and as such is subject to the laws of inorganic reaction, independent of its organic matrix. Various experimental data which indicate possible fallacies in this theory are discussed.

The second theory has been evolved around studies of the organic matrix. New histochemical methods permit the preservation of the organic matrix of enamel during the decalcification process so that sufficient matrix remains to be sectioned and stained for histologic examination. The working hypothesis given by Nuckolls and Frisbie as a result of these studies is "with the establishment of the lesion, caries is primarily a degradation of the organic matrix resulting from the enzymatic action of microorganisms which is followed by a subsequent physical disintegration of the inorganic salts." The authors conclude "no single bit of evidence or critical analysis of the experimental work on caries points to the early solution of the problem."

The Growth Promoting Factor of Summer Butter

Some years ago the existence of a growth promoting factor present in butter produced when the cows were kept on pasture ("summer butter") but not when they were maintained indoors on winter rations ("winter butter") was announced independently by E. J. Schantz, R. K. Boutwell, C. A. Elvehjem, and E. B. Hart (*J. Dairy Sci.* **23,** *181, 1205 (1940)*) and by J. Boer and B. C. P. Jansen (*Arch. néerland. de physiol.* **26,** *1 (1942)*).

The Dutch workers have been able to continue their investigations through the war years and have now made a preliminary announcement of the isolation of this factor (J. Boer, B. C. P. Jansen, and A. Kentie, *Nature* **158,** *201 (1946)*). The active fraction of the summer butter has been traced to the saponifiable portion, hence this portion was further fractionated by distillation into a series of fatty acid fractions. These fractions were submitted to biologic test on a basal diet in which the fat used was rapeseed oil. A fraction consisting almost entirely of vaccenic acid (Δ^{11}-octadecylenic acid, an isomer of oleic acid) was found to abolish the difference between growth rates on the basal diet and on the basal diet with added summer butter respectively.

The results showed a statistically significant effect due to the vaccenic acid and according to the authors establish this substance as the growth promoting factor of summer butter.

Tryptophane

A brief (16 page) review on "Recent Researches on Tryptophane" has been prepared by the Special Chemicals Division of Winthrop Chemical Company, Inc., Rensselaer, New York. A rather complete list of more than one hundred and fifty references to research reports on this subject are included.

Recent Nutrition Books

Hunger Osteomalacie in Holland (Hongerosteopathie in Nederland). A. W. M. Pompen, E. H. La Chapelle, J. Groen, and K. P. M. Mercx. Wetenschappelijke Uitgeverij, Amsterdam, Holland. 1946. Pp. 107.

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